Figure 1a

```
   1 GCGGCCGCGT CGACCCGGCG TTCAGACGCG GGCAGCTACC GGCGCTCGCT GGGCTCCGCG
  61 GGGCCGTCGG GCACTTTGCC TCGCAGCTGG CAGCCCGTCA GCCGCATCCC CATGCCCCCC
 121 TCCAGCCCCC AGCCCCGCGG GGCCCCGCGC CAGCGTCCCA TCCCCCTCAG CATGATCTTC
 181 AAGCTGCAGA ACGCCTTCTG GGAGCACGGG GCCAGCCGCG CCATGCTCCC TGGGTCCCCC
 241 CTCTTCACCC GAGCACCCCC GCCTAAGCTG CAGCCCCAAC CACAACCACA GCCCCAGCCA
 301 CAATCACAAC CACAGCCCCA GCTGCCCCAA CAGCCCCAGA CCCAACCCCA AACCCCTACC
 361 CCAGCCTCCC ACATCCGCAT CCCCAACAGA CATGGCCCCC TGTGAACGAA GGACCCCCCA
 421 AACCCCCCAC CGAGCTGGAG CCTGAGCCGG AGATAGAGGG GCTGCTGACA CCAGTGCTGG
 481 AGGCTGGCGA TGTGGATGAA GGACCCTGTA GCAAGGCCTC TCAGCCCCAC GAGGCTGCAG
 541 CCAGCACTGC CACCGGAGGC ACAGTCGGTG CCCGAGCTGG AGGAGGTGGC ACGGGTGTTG
 601 GCGGAAATTC CCCGGCCCCT CAAACGCAGG GGCTCCATGG AGCAGGCCCC TGCTGTGGCC
 661 CTGCCCCCTA CCCACAAGAA ACAGTACCAG CAGATCATCA GCCGCCTCTT CCATCGTCAT
 721 GGGGGGCCAG GGCCCGGGGG GCGGAGCCAG AGCTGTCCCC CATCACTGAG GGATCTGAGG
 781 CCAGGGCAGG GCCCCCTGCT CCTGCCCCAC CAGCTCCCAT TCCACCGCCC GGCCCCGTCC
 841 CAGAGCAGCC CACCAGAGCA GCCGCAGAGC ATGGAGATGC GCTCTGTGCT GCGGAAGGCG
 901 GGCTCCCCGC GCAAGGCCCG CCGCGCGCGC CTCAACCCTC TGGTGCTCCT CCTGGACGCG
 961 GCGCTGACCG GGGAGCTGGA GGTGGTGCAG CAGGCGGTGA AGGAGATGAA CGACCCGAGC
1021 CAGCCCAACG AGGAGGGCAT CACTGCCTTG CACAACGCCA TCTGCGGCGC CAACTACTCT
1081 ATCGTGGATT TCCTCATCAC CGCGGGTGCC AATGTCAACT CCCCCGACAG CCACGGCTGG
1141 ACACCCTTGC ACTGCGCGGC GTCGTGCAAC GACACAGTCA TCTGCATGGC GCTGGTGCAG
1201 CACGGCGCTG CAATCTTCGC CACCACGCTC AGCGACGGCG CCACCGCCTT CGAGAAGTGC
1261 GACCCTTACC GCGAGGGTTA TGCTGACTGC GCCACCTACC TGGCAGACGT CGAGCAGAGT
1321 ATGGGGCTGA TGAACAGCGG GGCAGTGTAC GCTCTCTGGG ACTACAGCGC CGAGTTCGGG
1381 GACGAGCTGT CCTTCCGCGA GGGCGAGTCG GTCACCGTGC TGCGGAGGGA CGGGCCGGAG
1441 GAGACCGACT GGTGGTGGGC CGCGCTGCAC GGCCAGGAGG GCTACGTGCC GCGGAACTAC
1501 TTCGGGCTGT TCCCCAGGGT GAAGCCTCAA AGGAGTAAAG TCTAGCAGGA TAGAAGGAGG
1561 TTTCTGAGGC TGACAGAAAC AAGCATTCCT GCCTTCCCTC CAGACCTCTC CCTCTGTTTT
1621 TTGCTGCCTT TATCTGCACC CCTCACCCTG CTGGTGGTGG TCCTTGCCAC CGGTTCTCTG
1681 TTCTCCTGGA AGTCCAGGGA AGAAGGAGGG CCCCAGCCTT AAATTTAGTA ATCTGCCTTA
1741 GCCTTGGGAG GTCTGGGAAG GGCTGGAAAT CACTGGGGAC AGGAAACCAC TTCCTTTTGC
1801 CAAATCAGAT CCCGTCCAAA GTGCCTCCCA TGCCTACCAC CATCATCACA TCCCCCAGCA
1861 AGCCAGCCAC CTGCCCAGCC GGGCCTGGGA TGGGCCACCA CACCACTGGA TATTCCTGGG
1921 AGTCACTGCT GACACCATCT CTCCCAGCAG TCTTGGGGTC TGGGTGGGAA ACATTGGTCT
1981 CTACCAGGAT CCCTGCCCCA CCTCTCCCCA ATTAAGTGCC TTCACACAGC ACTGGTTTAA
2041 TGTTTATAAA CAAAATAGAG AAACTGGTTT AATGTTTATA AACAAAATAG AGAAACTTTC
2101 GCTTATAAAT AAAAGTAGTT TGCACAGAAA TGAAAAAAAA AAAAAAAAAA AAAAAA
```

Figure 1b

```
   1 atggtcacga ccagtagcgg agggggtata gggtacccgg caaacaacgg tgtcacacag
  61 gtgtctctga ttcactcgtc ggattctgta cgaactgttt caactgcccc aatataccgt
 121 ccgacgtcat caatggcatc tacgatggct cataaatctt cgacggctcc gttcatctcc
 181 gcaaatcaac gaatgtcaaa accgccggtt cgggtggtcg ctcaaccacc accaccacat
 241 ccacaagcat tgtcccaaca gtatcaccag cagaatccga tgatgatgta ttccgcacca
 301 aatacacgac cacacgttat tccgacaatg caagtgcaac cgacaatggc cgctcaaatt
 361 aaacgaaata atcctgttaa tgcacagttt cagaaccctt ctgaaatgat cgccgattac

 421 ggtgtaaaac cgcagtcagt agaaatggtg caaagagttc gagctgttcg aagacaagtc
 481 gccgacgagg agaccgaact gcgaagactc agagagcttg aacacgaaac ggcacagctt
 541 caaaataaga attatggaag agaaagagag ttgaatgtgc aaggatccat gctgaaagaa
 601 gctcaattag agttgagaaa tgcttcaatg agggcgcaat ctttaaacaa gcatttggaa
 661 gaaatgtacc ggagaagaca aactgcagca gcggcagcgc tcgtggaaca acgaaaaatg
 721 cagcaacatc agattcttct agcccgagct gcaaatcaag tatccacaca agaagttata
 781 agacctcgtg cttctgtcga accattccaa gttaataata cccaacagca acaaccatca
 841 cctcaaatga tgaaatcaga agaattttcg gagaaaagag atttgaatgg acaaactggc
 901 agttatgatg ctatcgatgg atcaggagat catcaaaaaa taccgacgga gccatcgtac
 961 ttggcaccat gtaaagaaaa ccagcaaaaa tactcggagt taagtaaaat ggcatctacg
1021 gatcctcatt caaatcacag ttcaccatca acttcttcgc agaaagctcc gacgttgatc
1081 acatttttctc caccaagttt tgaacagaaa atcaactcgt ctacaatgac tcgggattct
1141 ccgttcgttg agcgtccaac atcgtttggt gatagtctag acgaatcacg actgagaagt
1201 ggaaagactg atttggtatc acttcgatca gattcgctga aagctacgaa acgtcgttct
1261 tgggctgctt ccgaaggtac ttcaatgtca gaggcagaga tgattcatag gcttcttgat
1321 gaacaacgtc gtgggagatc acattttatt ccacaattgc caacatcaca agaagaacca
1381 tcggcaataa catcagaaac atatgccgaa gaagttgtca attcagaatc gaaacaagtt
1441 gctacaagtt cggattccac taataatctt gaattgccaa ccgaacaaat ggtattaggt
1501 agtgatacca caacagaaga agatgcaagt tcgtgttcaa cacgttctga tgatggacag
1561 aatcttgaaa tggaagttgc gattgaaaga agaactgtta aaggcatttt gagaagacct
1621 aatgaaaaga tgaacaaagg tcgcattgaa tttgacccat tagcactctt gctcgatgct
1681 gctttagaag gagaactcga tttagtgaga agcagtgcct caaagctaac agatgtctca
1741 caggccaatg atgaagggat tacggcgttg cacaatgcga tttgtgctgg acactatgag
1801 attgtaagat ttttgatcga gaacgacgct gatgtgaatg ctcaagattc cgatggttgg
1861 actccacttc attgtgcagc ttcctgtaat aaccttccaa tggttagaca acttgtggaa
1921 ggaggaggat gcgttctcgc ttcgacacta tctgatatgg aaacacctgt ggagaagtgt
1981 gaagaagatg aagatggtta tgatggatgt ttgaagtatc tttccgcagc ccataactca
2041 acgggatcaa ttaatactgg aaaagtttac gctgcttatg gatatgaggc ggcatttgaa
2101 gatgagctca gttttgatgc aggagatgaa ttgacggtta ttgagaaaga taaagtcgat
2161 aaaaattggt ggacatgtga gaagaacaat ggagagaagg gacaagtacc aagaacatat
2221 ttggcgttgt acccatcgtt aaaatacaga aagaagctca actttgtgat gttcgatctt
2281 ccattggaat cgaacaacaa tgtcgaataa
```

Figure 2a

MWMKDPVARPLSPTRLQPALPPEAQSVPELEEVARVLAEIPRPL

KRRGSMEQAPAVALPPTHKKQYQQIISRLFHRHGGPGPGGRSQSCPPSLRDLRPGQGP

LLLPHQLPFHRPAPSQSSPPEQPQSMEMRSVLRKAGSPRKARRARLNPLVLLLDAALT

GELEVVQQAVKEMNDPSQPNEEGITALHNAICGANYSIVDFLITAGANVNSPDSHGWT

PLHCAASCNDTVICMALVQHGAAIFATTLSDGATAFEKCDPYREGYADCATYLADVEQ

SMGLMNSGAVYALWDYSAEFGDELSFREGESVTVLRRDGPEETDWWWAALHGQEGYVP

RNYFGLFPRVKPQRSK

Figure 2b

MVTTSSGGGIGYPANNGVTQVSLIHSSDSVRTVSTAPIYRPTSS.
MASTMAHKSSTAPFISANQRMSKPPVRVVAQPPPPHPQALSQQYHQQNPMMMYSAPNT
RPHVIPTMQVQPTMAAQIKRNNPVNAQFQNPSEMIADYGVKPQSVEMVQRVRAVRRQV
ADEETELRRLRELEHETAQLQNKNYGRERELNVQGSMLKEAQLELRNASMRAQSLNKH
LEEMYRRRQTAAAAALVEQRKMQQHQILLARAANQVSTQEVIRPRASVEPFQVNNTQQ
QQPSPQMMKSEEFSEKRDLNGQTGSYDAIDGSGDHQKIPTEPSYLAPCKENQQKYSEL
SKMASTDPHSNHSSPSTSSQKAPTLITFSPPSFEQKINSSTMTRDSPFVERPTSFGDS
LDESRLRSGKTDLVSLRSDSLKATKRRSWAASEGTSMSEAEMIHRLLDEQRRGRSHFI
PQLPTSQEEPSAITSETYAEEVVNSESKQVATSSDSTNNLELPTEQMVLGSDTTTEED
ASSCSTRSDDGQNLEMEVAIERRTVKGILRRPNEKMNKGRIEFDPLALLLDAALEGEL
DLVRSSASKLTDVSQANDEGITALHNAICAGHYEIVRFLIENDADVNAQDSDGWTPLH
CAASCNNLPMVRQLVEGGGCVLASTLSDMETPVEKCEEDEDGYDGCLKYLSAAHNSTG
SINTGKVYAAYGYEAAFEDELSFDAGDELTVIEKDKVDKNWWTCEKNNGEKGQVPRTY
LALYPSLKYRKKLNFVMFDLPLESNNNVE

Figure 3a

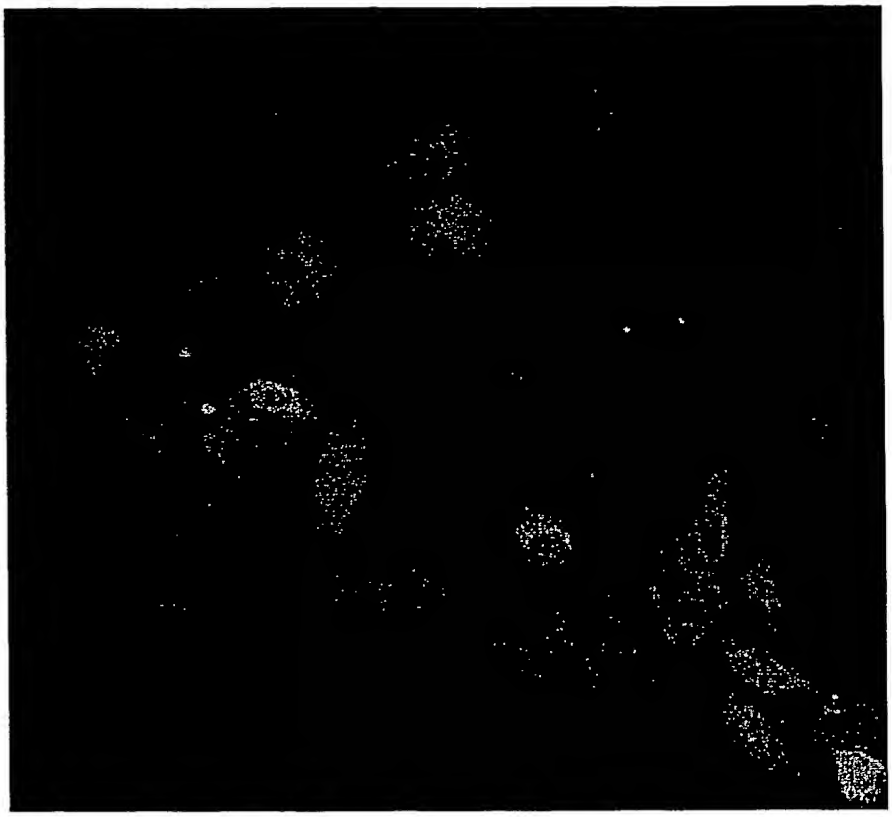


Figure 3 b

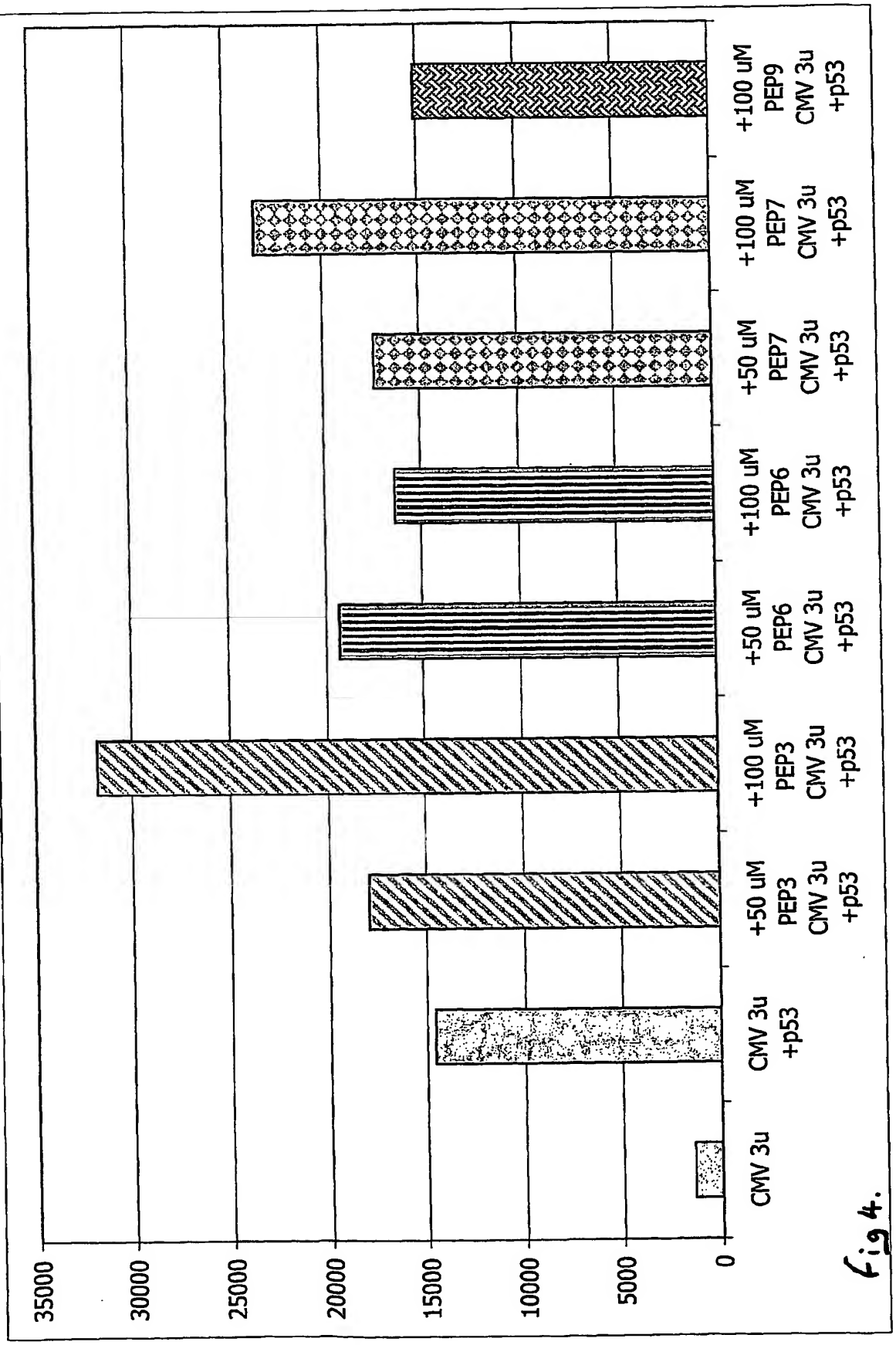

35000
30000
25000
20000
15000
10000
5000
0
CMV 3u
CMV 3u +p53
+50 uM PEP3 CMV 3u +p53
+100 uM PEP3 CMV 3u +p53
+50 uM PEP6 CMV 3u +p53
+100 uM PEP6 CMV 3u +p53
+50 uM PEP7 CMV 3u +p53
+100 uM PEP7 CMV 3u +p53
+100 uM PEP9 CMV 3u +p53

Fig 4.

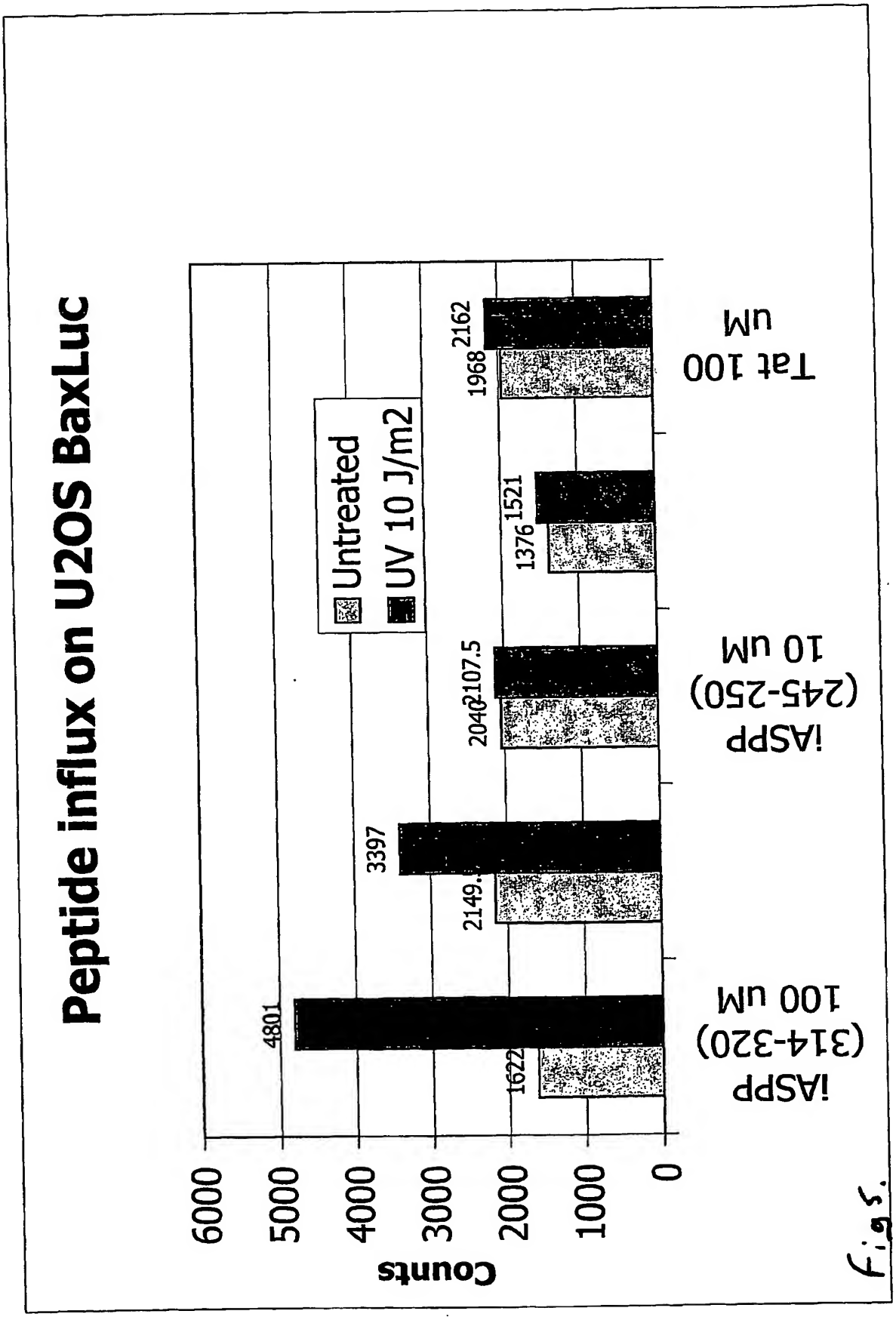

Peptide influx on U2OS BaxLuc
Counts
6000
5000
4000
3000
2000
1000
0
Untreated
UV 10 J/m2
4801
1622
3397
2149.
2040
2107.5
1376
1521
1968
2162
iASPP (314-320) 100 uM
iASPP (245-250) 10 uM
Tat 100 uM

Fig 5.

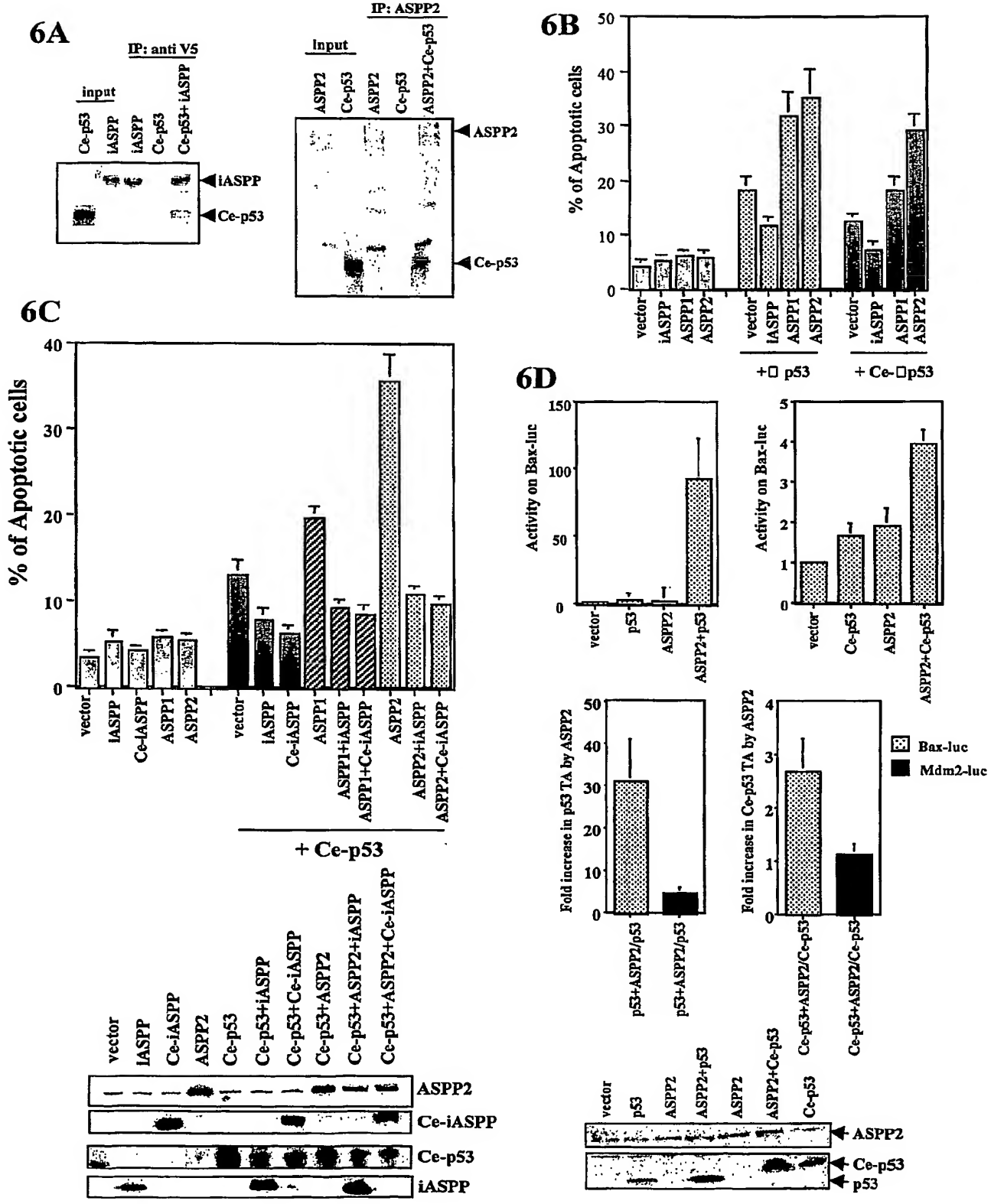
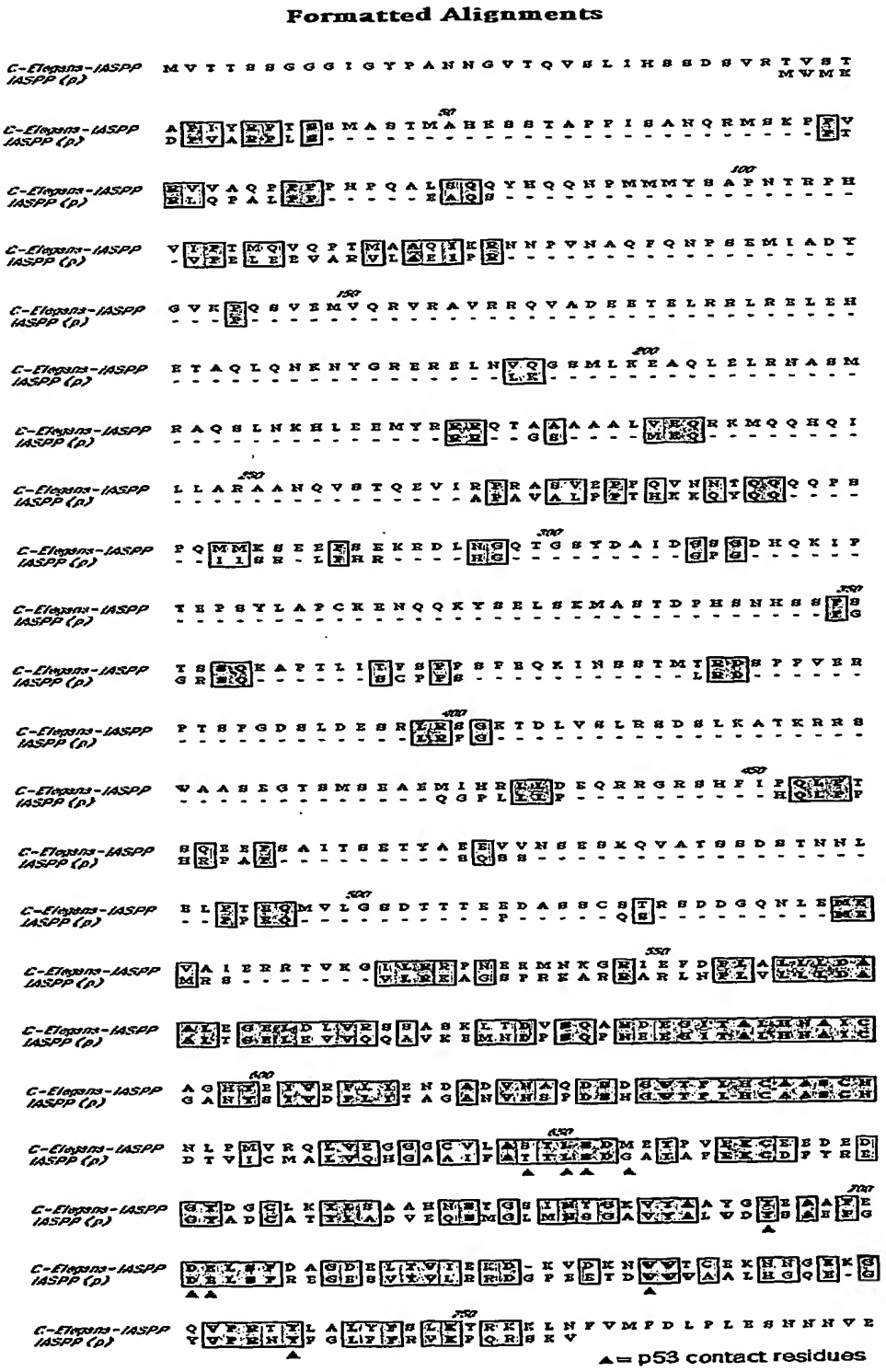
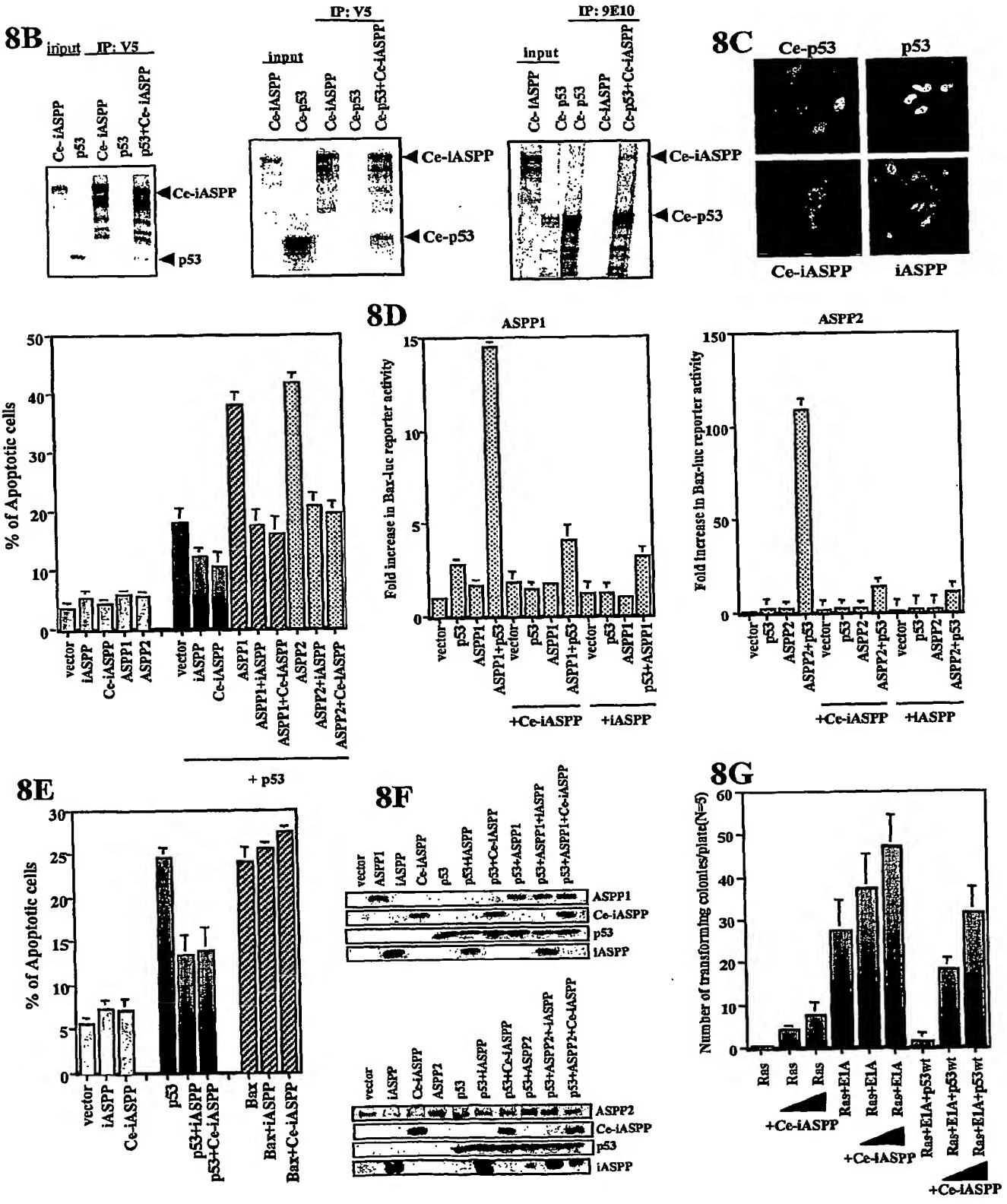

6A
IP: anti V5
input
Ce-p53
iASPP
iASPP
Ce-p53
Ce-p53+ iASPP
iASPP
Ce-p53
IP: ASPP2
Input
ASPP2
Ce-p53
ASPP2
Ce-p53
ASPP2+Ce-p53
ASPP2
Ce-p53
6B
% of Apoptotic cells
vector
iASPP
ASPP1
ASPP2
+□ p53
+ Ce-□p53
6C
% of Apoptotic cells
vector
iASPP
Ce-iASPP
ASPP1
ASPP2
ASPP1+iASPP
ASPP1+Ce-iASPP
ASPP2+iASPP
ASPP2+Ce-iASPP
+ Ce-p53
6D
Activity on Bax-luc
vector
p53
ASPP2
ASPP2+p53
Ce-p53
ASPP2+Ce-p53
Fold increase in p53 TA by ASPP2
Fold increase in Ce-p53 TA by ASPP2
p53+ASPP2/p53
Ce-p53+ASPP2/Ce-p53
Bax-luc
Mdm2-luc
Ce-iASPP
Ce-p53+iASPP
Ce-p53+Ce-iASPP
Ce-p53+ASPP2
Ce-p53+ASPP2+iASPP
Ce-p53+ASPP2+Ce-iASPP
ASPP2
Ce-iASPP
Ce-p53
iASPP
p53
ASPP2+p53
Ce-p53

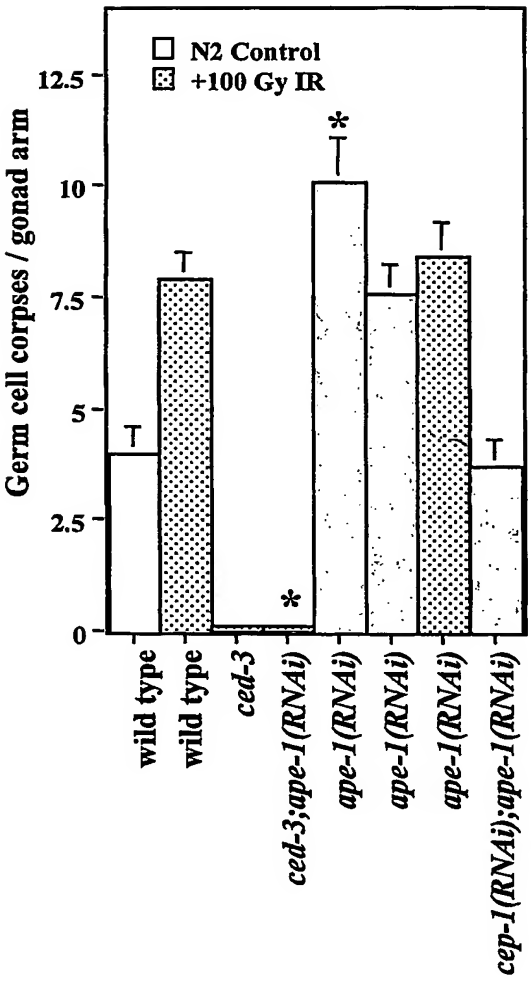
Figure 7


Formatted Alignments
C-Elegans-iASPP
iASPP (p)
▲= p53 contact residues


8B
input
IP: V5
Ce- iASPP
p53
Ce- iASPP
p53
p53+Ce- iASPP
Ce-iASPP
p53
input
IP: V5
Ce-iASPP
Ce-p53
Ce-iASPP
Ce-p53
Ce-p53+Ce-iASPP
Ce-iASPP
Ce-p53
input
IP: 9E10
Ce- iASPP
Ce- p53
Ce- p53
Ce-iASPP
Ce-p53+Ce-iASPP
Ce-iASPP
Ce-p53
8C
Ce-p53
p53
Ce-iASPP
iASPP
8D
% of Apoptotic cells
vector
iASPP
Ce-iASPP
ASPP1
ASPP2
vector
iASPP
Ce-iASPP
ASPP1
ASPP1+iASPP
ASPP1+Ce-iASPP
ASPP2
ASPP2+iASPP
ASPP2+Ce-iASPP
+ p53
ASPP1
Fold increase in Bax-luc reporter activity
vector
p53
ASPP1
ASPP1+p53
vector
p53
ASPP1
ASPP1+p53
vector
p53
ASPP1
p53+ASPP1
+Ce-iASPP
+iASPP
ASPP2
Fold increase in Bax-luc reporter activity
vector
p53
ASPP2
ASPP2+p53
vector
p53
ASPP2
ASPP2+p53
vector
p53
ASPP2
ASPP2+p53
+Ce-iASPP
+iASPP
8E
% of Apoptotic cells
vector
iASPP
Ce-iASPP
p53
p53+iASPP
p53+Ce-iASPP
Bax
Bax+iASPP
Bax+Ce-iASPP
8F
vector
ASPP1
iASPP
Ce-iASPP
p53
p53+iASPP
p53+Ce-iASPP
p53+ASPP1
p53+ASPP1+iASPP
p53+ASPP1+Ce-iASPP
ASPP1
Ce-iASPP
p53
iASPP
vector
iASPP
Ce-iASPP
ASPP2
p53
p53+iASPP
p53+Ce-iASPP
p53+ASPP2
p53+ASPP2+iASPP
p53+ASPP2+Ce-iASPP
ASPP2
Ce-iASPP
p53
iASPP
8G
Number of transforming colonies/plate(N=5)
Ras
Ras
Ras
Ras+E1A
Ras+E1A
Ras+E1A
Ras+E1A+p53wt
Ras+E1A+p53wt
Ras+E1A+p53wt
+Ce-iASPP
+Ce-iASPP
+Ce-iASPP

ape-1= iASPP
cep-1 = *Ce*-iASPP

9A



9B

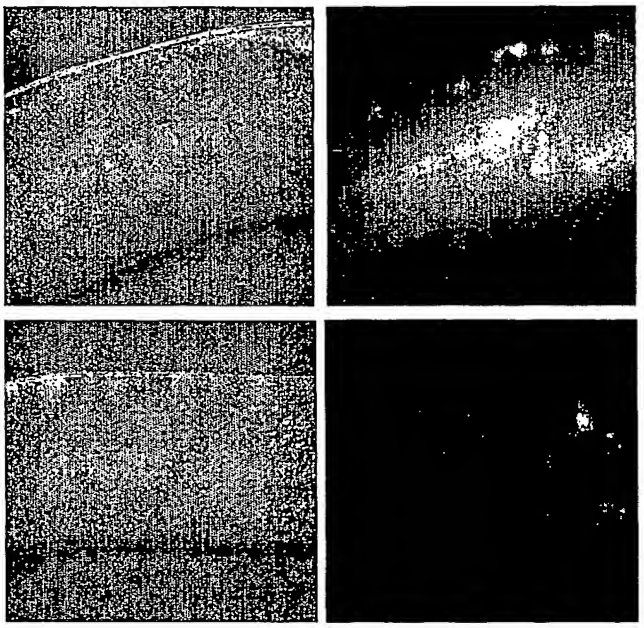


Figure 10

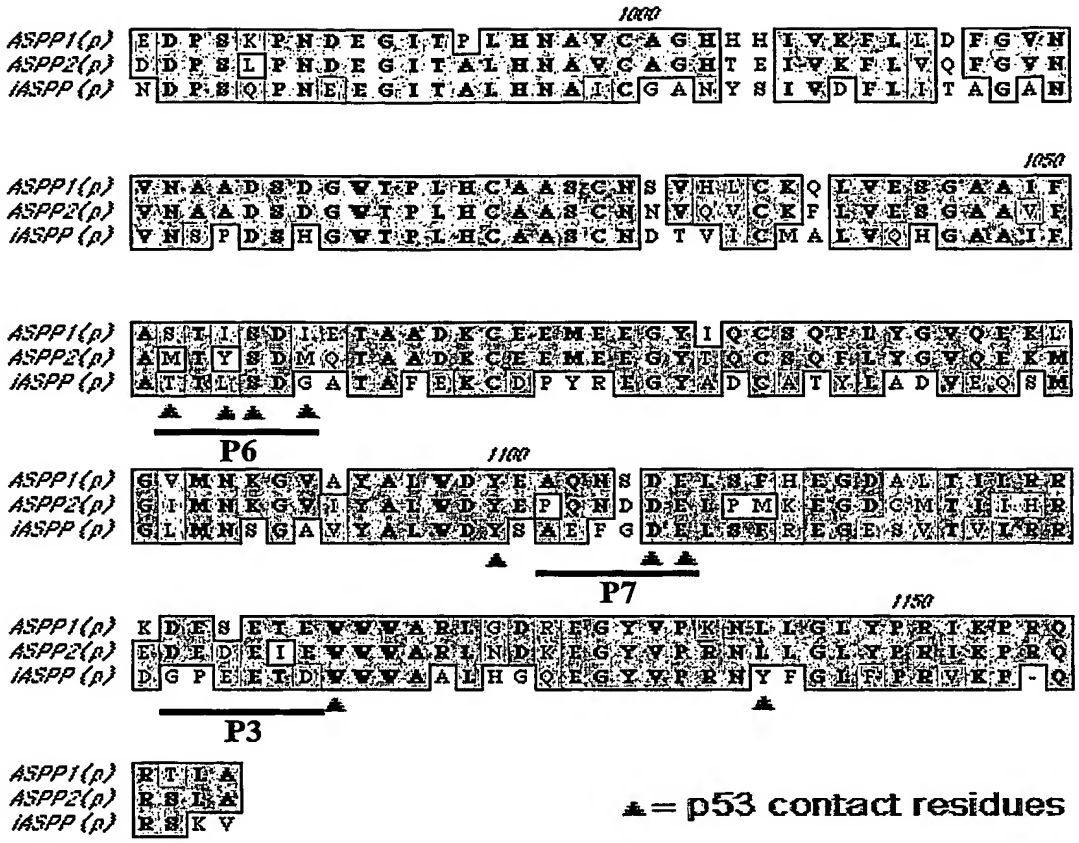

1000
ASPP1(p)
ASPP2(p)
iASPP(p)
1050
P6
1100
P7
1150
P3
= p53 contact residues

Figure 11

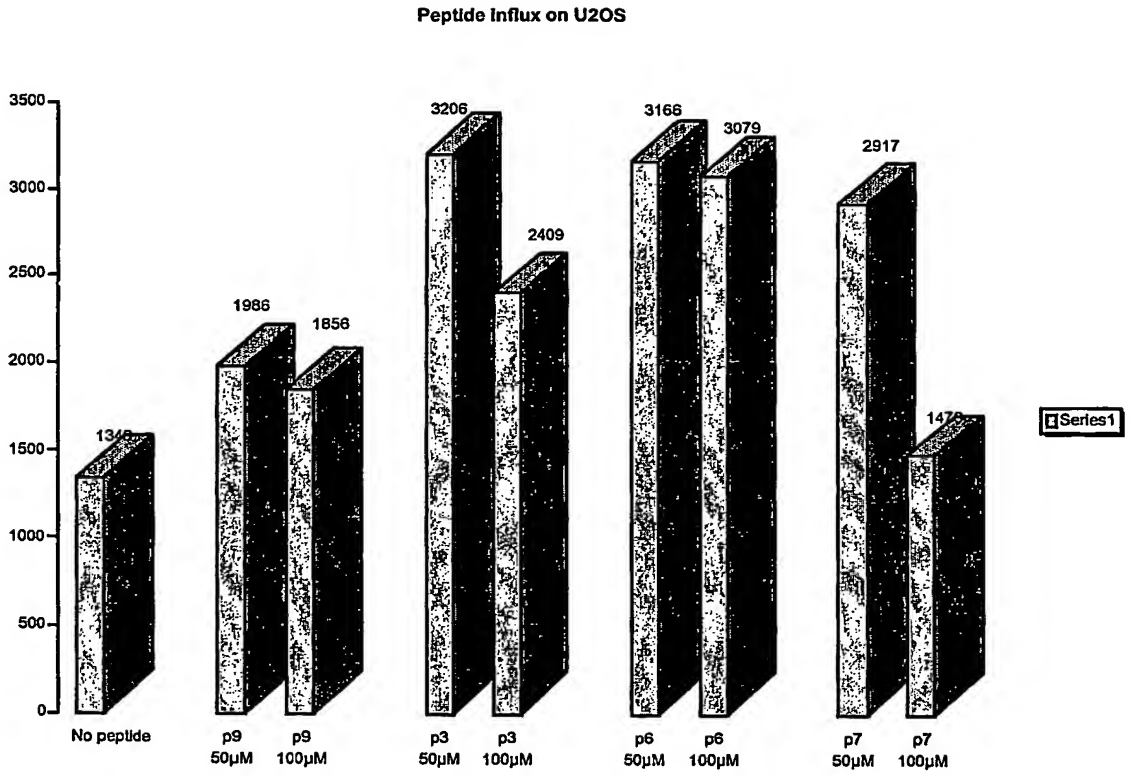

Peptide Influx on U2OS
3500
3000
2500
2000
1500
1000
500
0
1346
1986
1856
3206
2409
3166
3079
2917
1478
Series1
No peptide
p9 50µM
p9 100µM
p3 50µM
p3 100µM
p6 50µM
p6 100µM
p7 50µM
p7 100µM

Figure 12

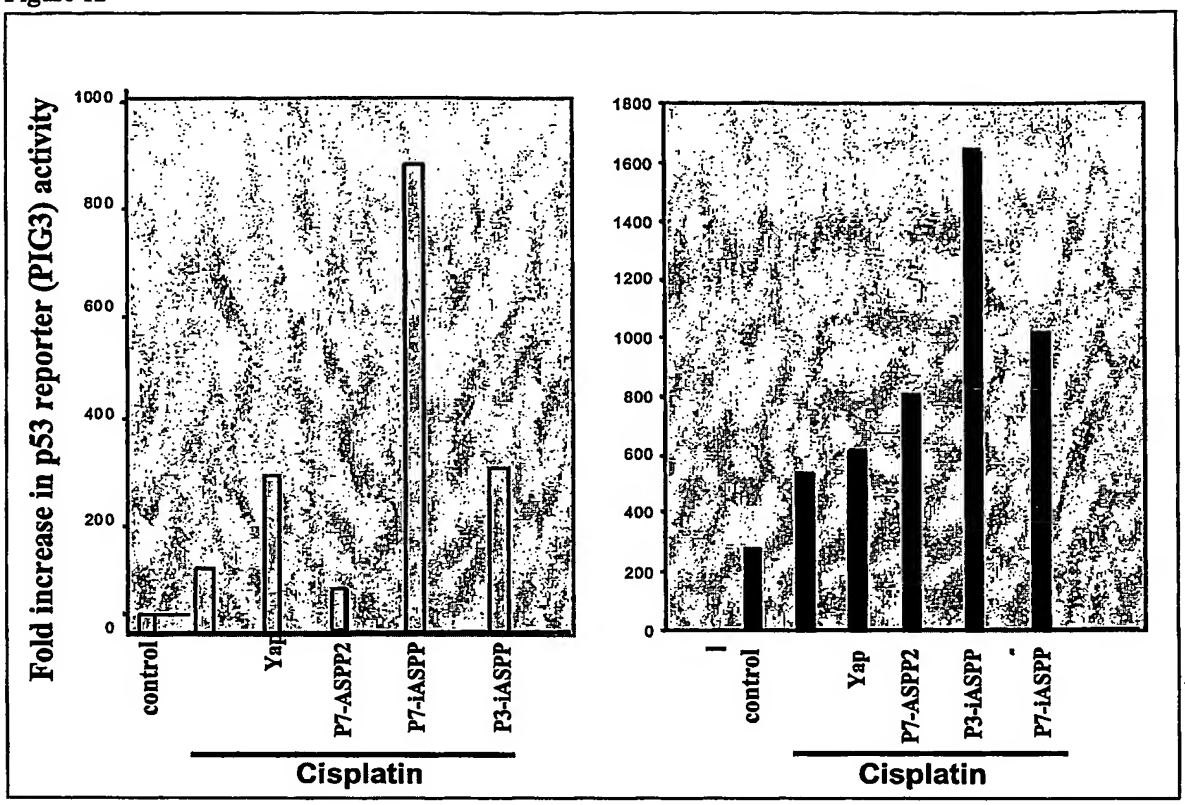
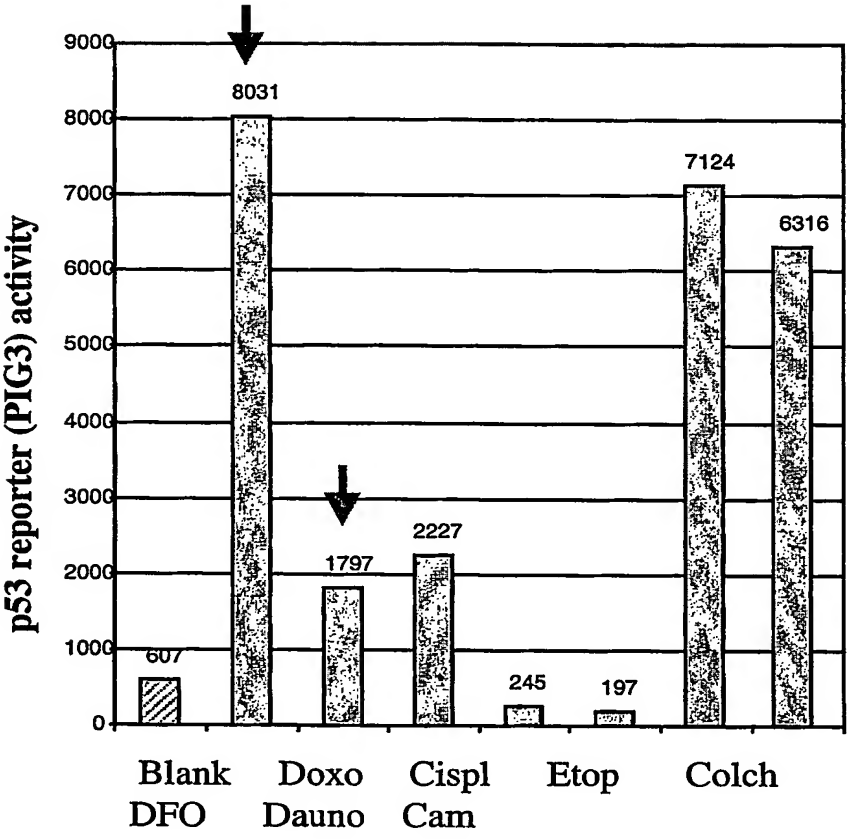
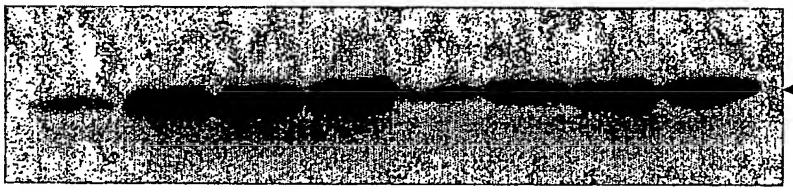

Fold increase in p53 reporter (PIG3) activity
1000
800
600
400
200
0
control
Yap
P7-ASPP2
P7-iASPP
P3-iASPP
Cisplatin
1800
1600
1400
1200
1000
800
600
400
200
0
control
Yap
P7-ASPP2
P3-iASPP
P7-iASPP
Cisplatin

Figure 13


p53 reporter (PIG3) activity
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
607
8031
1797
2227
245
197
7124
6316
Blank
DFO
Doxo
Dauno
Cispl
Cam
Etop
Colch


p53

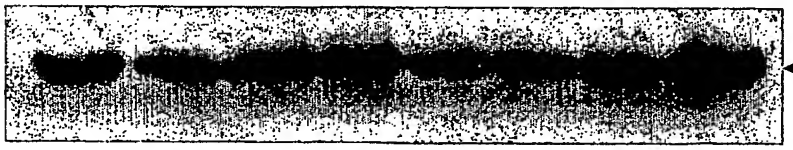

PCNA

Figure 14a

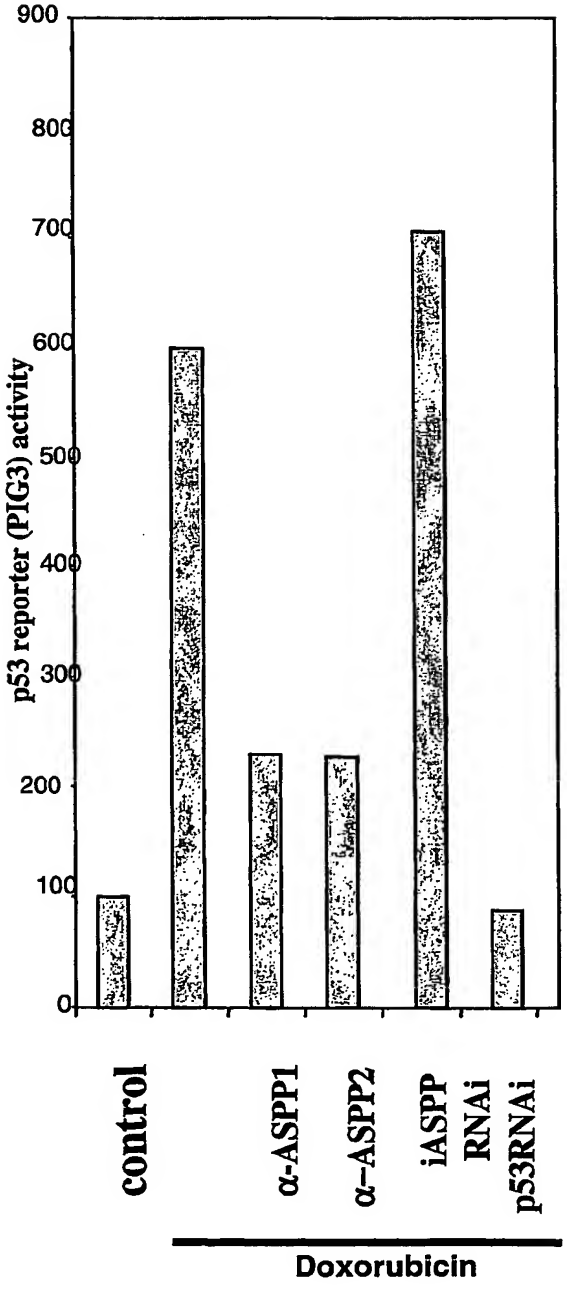


Figure 14b

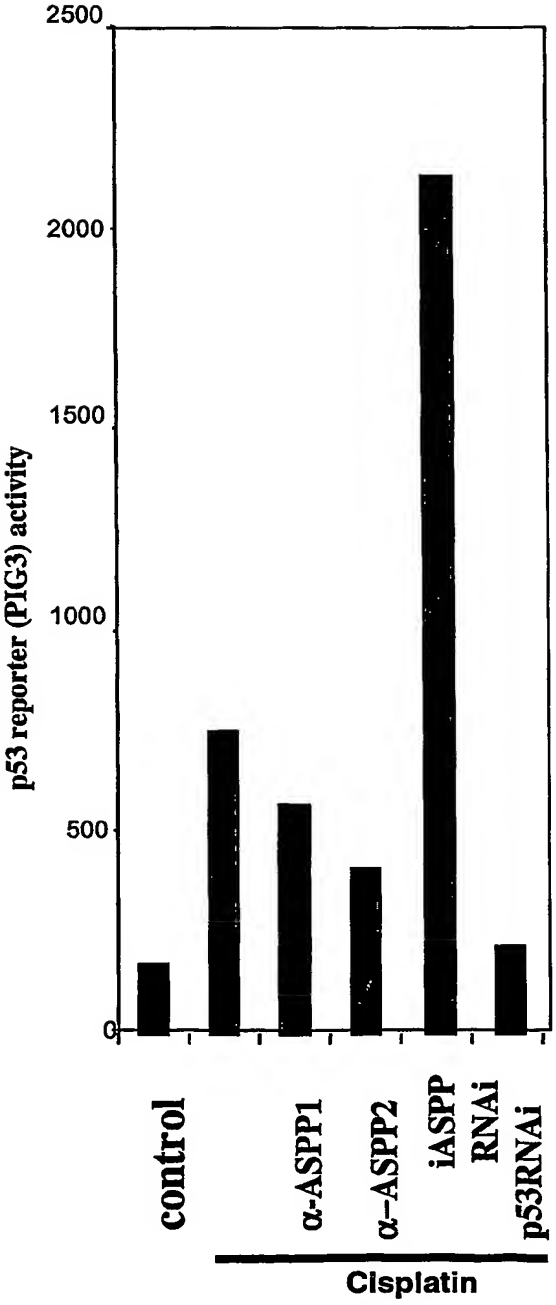
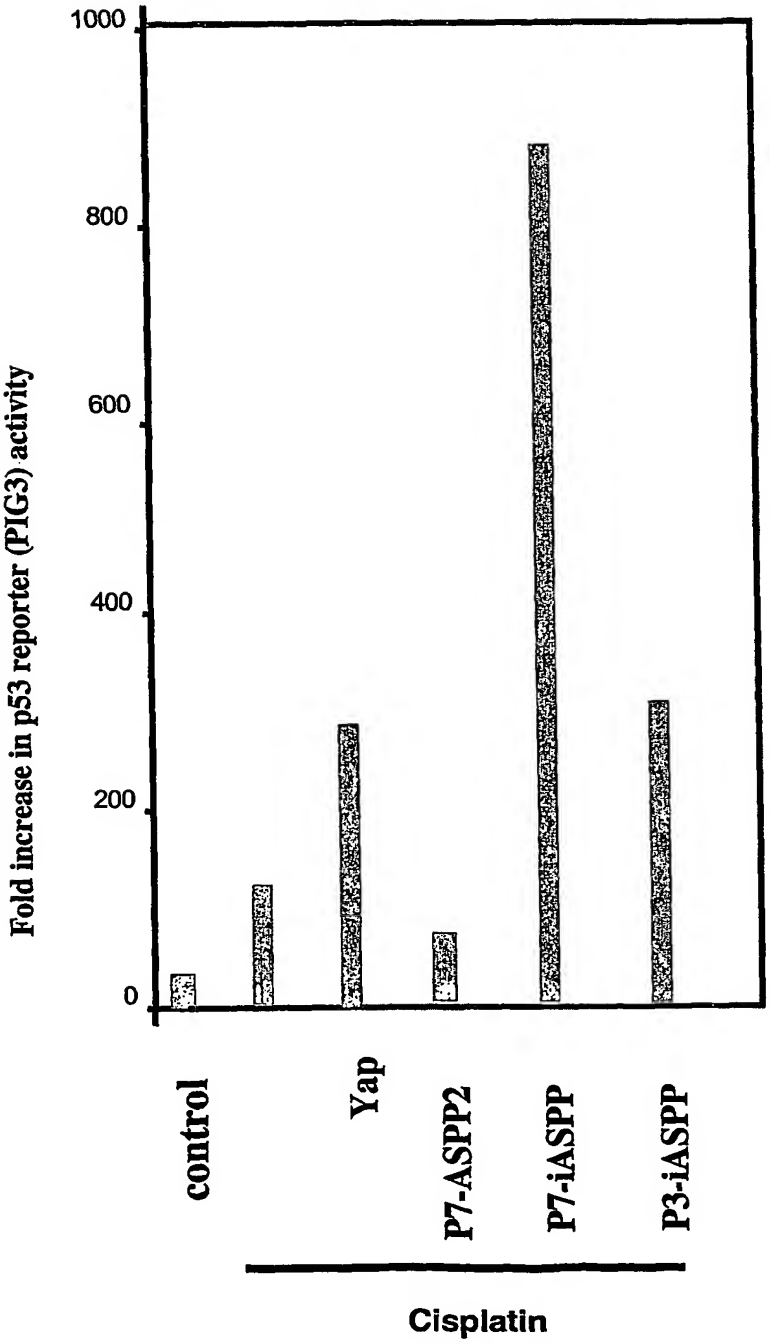


Figure 15



P7-ASPP2: PQNDDE
P7-iASPP: AEFGDE

P3-iASPP: DGPEETD

Table 1

	Human	Mouse	C.El.	Drosophila	Fugu I	Fugu II	Fugu III	Fugu IV
Human (352)	X	X	X	X	X	X	X	X
Mouse (260)	93.7	X	X	X	X	X	X	X
C.El. (769)	20.4	38.8	X	X	X	X	X	X
Drosophila (1071)	43.2	40.0	32.4	X	X	X	X	X
Fugu I (260)	51.9	45.0	48.1	55.4	X	X	X	X
Fugu II (252)	54.8	54.8	51.6	58.7	X	X	X	X
Fugu III (144)	54.2	53.5	54.2	64.6	X	X	X	X
Fugu IV (132)	51.5	50.8	55.3	62.9	X	X	X	X